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Two new species of the genus *Cyrtarachne* (Araneae: Araneidae) from Japan hitherto identified as *C. inaequalis*

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Abstract — After examining many specimens identified as *Cyrtarachne inaequalis* Thorell 1895 from various localities in Japan, females from Okinawajima Is. were found to be morphologically distinguishable from those from the Mainland and to be the independent species, while unidentified male specimens from Okinawajima Is. were found. A comparison of mt-DNA COI gene sequencing data revealed that the males and females from Okinawajima Is. belonged to the same species. The female specimens from the Mainland and Okinawajima Is. were compared with syntypes of *C. inaequalis*, but neither of the two species could be identified using this approach. Consequently, it was concluded that both of these species are new to science. They are described under the names *C. akirai* n. sp. (from the Mainland) and *C. jucunda* n. sp. (from Okinawajima Is.).

Key words — taxonomy, *Cyrtarachne akirai*, *Cyrtarachne jucunda*, COI, DNA

Introduction

Cyrtarachne inaequalis Thorell 1895 was first described in Burma and has been recorded in India, China, Korea and Japan (Platnick 2013). Its distribution range is the widest among the four Japanese congeners, since it has been recorded in Honshu, Shikoku, Kyushu, and Nansei Isls., but not in Hokkaido (Shinkai et al. 2012). Some spiders with a wide distribution range, especially from tropical to temperate regions in Japan, were misidentified as several independent species (Tanikawa 1994, Chida & Tanikawa 1999, Tanikawa et al. 2010).

After an examination of many specimens collected from various parts of Japan and identified as *C. inaequalis*, it became apparent that two species have been confused. The female specimens from Okinawajima Is. can be distinguished from those of other regions by the shape of the epigyne, although the general appearances are quite similar. In this study, syntypes of *C. inaequalis* were examined to clarify the identity of the real inaequalis. In contrast, several unknown male specimens were collected from Okinawajima Is. Although these seemed to belong to the same species as the females from Okinawajima Is., it was impossible to determine the male specimens of the *Cyrtarachne* species using their general appearance due to their extreme sexual dimorphism. DNA sequencing analysis is quite useful for identification in such cases (Tanikawa et al. 2006, 2008; Tanikawa 2011) and was used to identify these unknown male specimens.

Materials and method

The specimens used for morphological study were preserved in 75% ethanol at room temperature. The morphological characters were examined under stereoscopic microscopes, M3Z (Wild Heerbrugg AG, Heerbrugg, Switzerland) and SZH (Olympus Corp., Tokyo, Japan), photographs were taken by EOS D60 with MP-E65 mm or EF100 mm macro lens and MT-24EX macro twin flash (Canon Inc., Tokyo, Japan). Details of specimens examined are shown in description part. All measurements are given in mm.

The specimens used for molecular work (see Appendix) were preserved in 75% or 99.5% ethanol at room temperature or 4°C. The genomic DNA was extracted from muscle of legs of female specimens and the whole cephalothorax of male specimens using DNeasy Blood & Tissue kit (Qiagen, Inc., Germantown, MD). The mitochondrial DNA cytochrome oxidase subunit I (mt-COI) partial sequence was amplified using the primer combination LCOI-1498: 5' – GGT CAA CAA ATC ATA AAG ATA TTG G – 3' with HCOI-2198: 5' – TAA ACT TCA GGG TGA CCA AAA AAT CA – 3' (Folmer et al. 1994). The reactants were initially denatured for 2 min at 94°C, proceeded with 40 cycles of 15 sec at 94°C, 20 sec at 47°C, 30 sec at 72°C. PCR product was purified using the ExoSAP-IT (GE Healthcare Bio-Sciences, Co. Ltd., Buckinghamshire, England). The purified PCR product was sequenced using the BigDye terminator cycle sequencing kit (ver. 3.1, Applied Biosystems, Foster City, CA) and analysed on ABI 3100 automated DNA sequencer (Applied Biosystems, Foster City, CA).

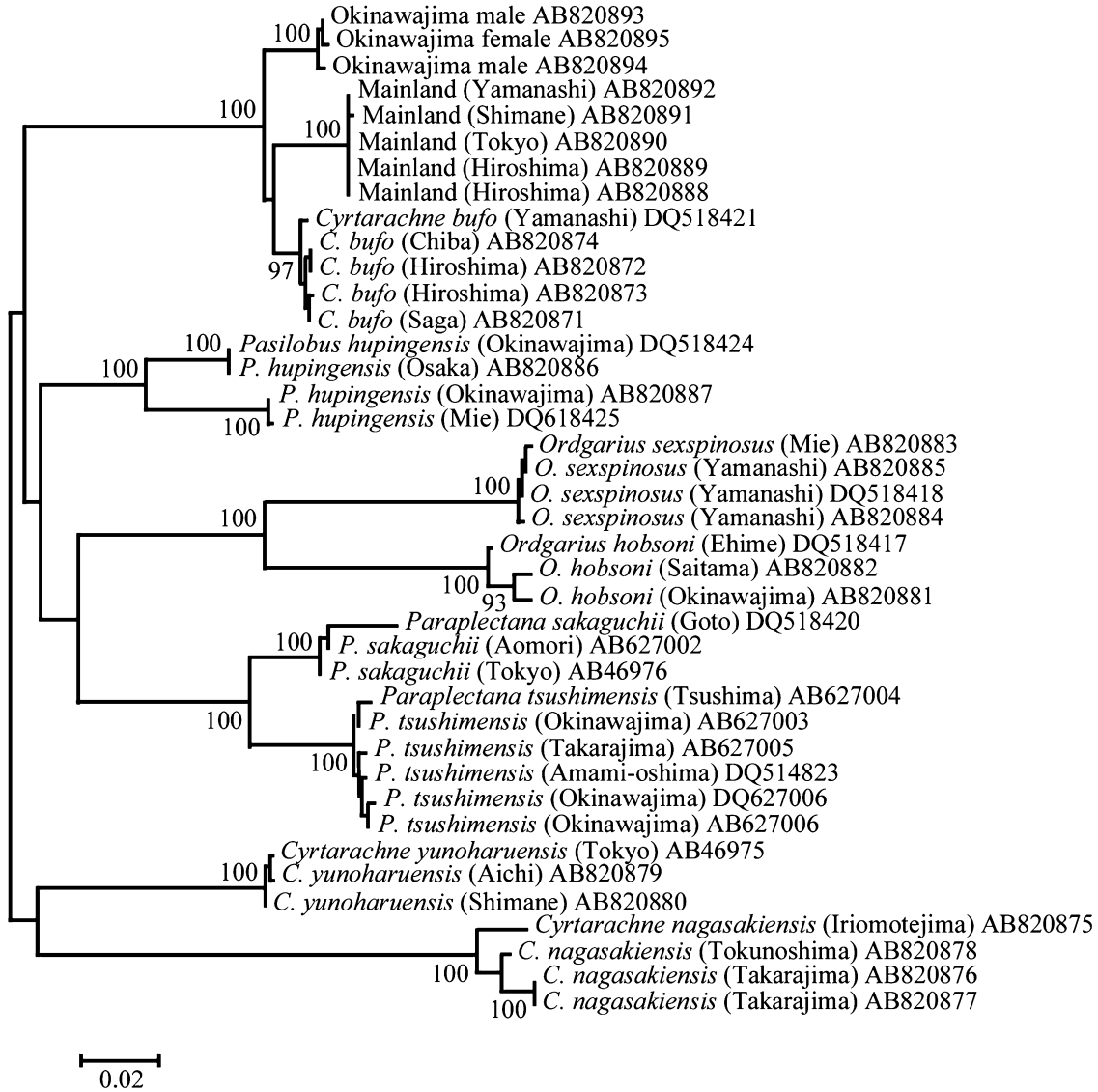


Fig. 1. Unrooted bootstrap consensus tree obtained by Neighbor Joining method for the species examined. Scores at each node are bootstrap values (1000 replicates, less than 90 omitted). Scale bar shows substitution per site.

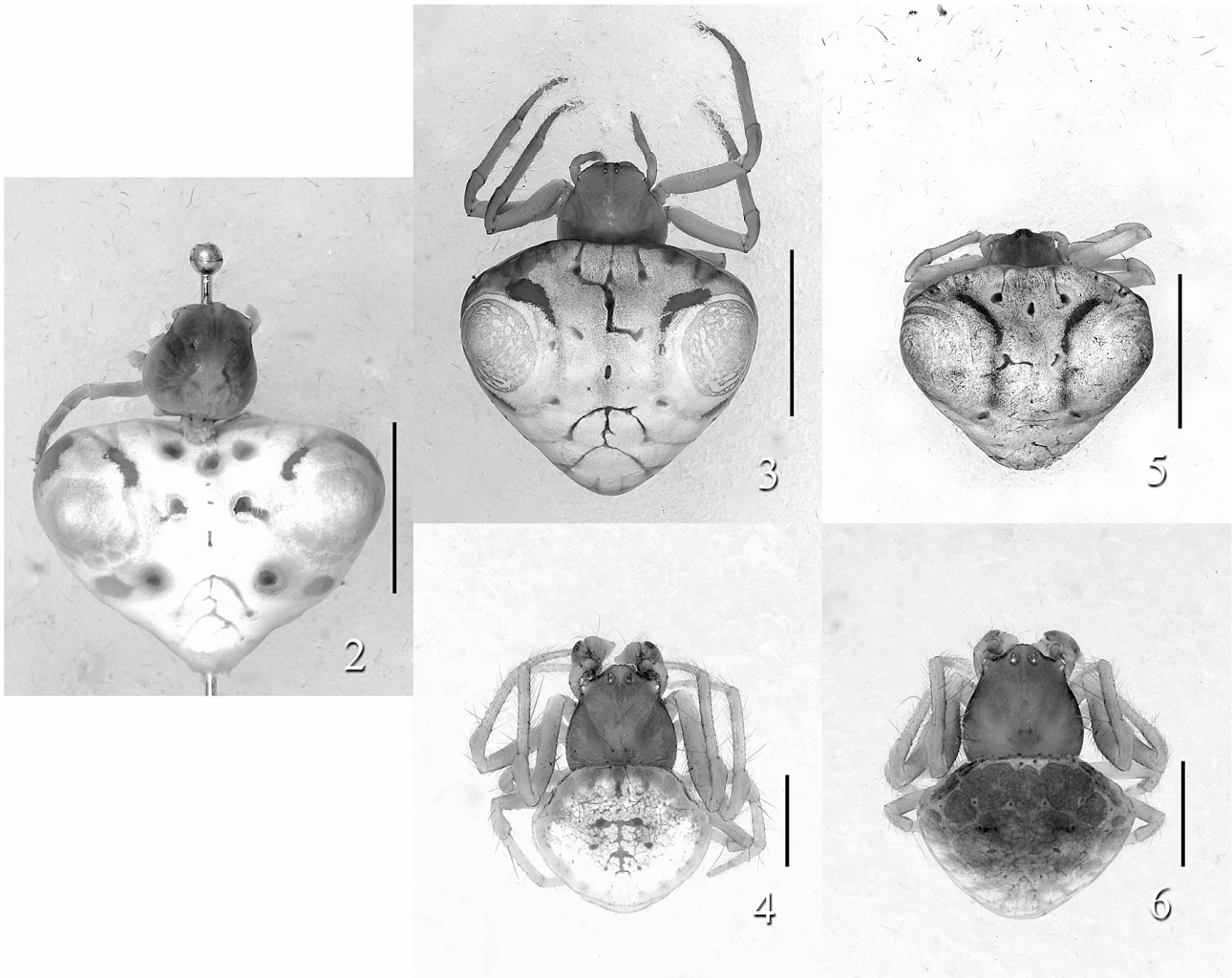
Chromatogram was checked by eye using MEGA version 5.05 (Tamura et al. 2011). The obtained sequences were compared with those of allied species, including those in DDBJ/EMBL/GenBank database (see Appendix). Sequence alignments were done by MUSCLE (Edgar 2004) in MEGA. The phylogenetic tree was constructed by Neighbor Joining method using MEGA.

Nucleotide sequence data are available in the DDBJ/EMBL/GenBank databases. The type specimens designated in this study are deposited in the collection of the Department of Zoology, National Museum of Nature and Science, Tokyo (NSMT).

Results and conclusion

The syntypes of *C. inaequalis* are one female (Fig. 2) and one juvenile. These resemble Japanese specimens from the Mainland (Fig. 3) and Okinawajima Is. in general appearance (Fig. 5), but can be clearly distinguished from one another by the shape of the epigyne. The female syntype has a wide and short scape (Figs. 7–8) that is much longer in the Mainland specimens (Figs. 9–10) and tongue-shaped in the Okinawajima specimens (Figs. 11–12). Consequently, neither of the Japanese species could be identified as *C. inaequalis*.

Partial sequences (621 bp) of mt-COI were obtained from the specimens. Accession numbers are shown in the Appendix. The obtained unrooted NJ tree is shown in Fig. 1. The mt-COI sequencing data for unknown male specimens from Okinawajima Is. were quite similar to those for females from the same island (Fig. 1). The p-distances (the number of base differences per site) were as follows: among Okinawajima specimens, 0.0016 or 0.0031; among Mainland specimens, 0.0000 or 0.0016; among *C. bufo*, 0.0000 to 0.0047; between Okinawajima specimens and Mainland



Figs. 2–6. Habitus. 2, *Cyrtarachne inaequalis*, female (syntype: NHM 1895.9.21.654–655); 3, *C. akirai* n. sp., female (holotype: NSMT-Ar 12557); 4, same, male (paratype: NSMT-Ar 12562); 5, *C. jucunda* n. sp., female (holotype: NSMT-Ar 12567); 6, same, male (paratype: NSMT-Ar 12570). Scales＝5 mm (2, 3, 5); 1 mm (4, 6).

specimens, 0.0369 to 0.0407; and between Okinawajima specimens and *C. bufo*, 0.0224 to 0.0266. These data suggest that the males and females from Okinawajima Is. belong to the same species because the sequencing data for mt-COI are quite similar and they formed a clade independent from those of related Mainland specimens or *C. bufo*.

The Japanese specimens were also not identifiable as any of the known species of the genus. Consequently, it is concluded that the two species from the Mainland and Okinawajima Is., respectively, are new to science. Many studies have misidentified these species, as shown in the synonymy section. Among previous studies, only the identification in Tikader (1982) is correct, on the basis of published figures (Tikader 1982, figs. 266–269).

Description of new species

Cyrtarachne akirai n. sp.
[Japanese name: Oh-torinofun-damashi]
(Figs. 3–4, 9–10, 13)

Cyrtarachne inaequalis: Yaginuma 1960, p. 61, fig. 146; Yaginuma 1986, p. 109, fig. 58.1; Chikuni 1989, p. 82, fig. 57; Yin et al. 1997, p. 272, figs. 182 a–e; Song, Zhu & Chen 1999, p. 279, figs. 162O, 163A, K; Namkung 2002, p. 285, figs. 19.46 a–b; Kim & Kim 2002, p. 204, figs. 40, 122, 231; Namkung 2003, p. 287, figs. 19.46 a–b; Tanikawa 2007, p. 48, figs. 40–41, 455–456; Tanikawa 2009, p. 427, figs. 43–44. [nec Thorell 1895, MISIDENTIFICATION]

Type series. Holotype: ♀, Maioka-cho, Totsuka-ku, Yokohama-shi, Kanagawa Pref., Japan, 3-IX-1983, A. Tanikawa leg. (NSMT-Ar 12557). Paratypes: 1♀, same locality as the holotype, 28-VIII-1980, H. Iijima leg. (NSMT-

Ar 12558). 1♀, same locality as the holotype, 18-IX-1982, A. Tanikawa leg. (NSMT-Ar 12560). 1♀, Noba-cho, Konan-ku, Yokohama-shi, Kanagawa Pref., 25-VIII-1980, T. Ueno leg. (NSMT-Ar 12561). 2♂, Tokunaga, Izu-shi, Sizuoka Pref. 5-VIII-1995 (1♂: NSMT-Ar 12563), 6-VIII-1995 (1♂: NSMT-Ar 12562), A. Tanikawa leg. 2♂, Shimoda-shi, Shizuoka Pref. 14-VIII-1980 (1♂: NSMT-Ar 12565), 18-VIII-1981 (1♂: NSMT-Ar 12564), A. Tanikawa leg. 1♂, near Yanase Dam, Kinsha-cho-ogawayama, Shikoku-chuo-shi, Ehime Pref., 2-VIII-1994, Y. Ihara leg. (NSMT-Ar 12566). 1♀, near Ishitegawa Dam, Matsuyama-shi, Ehime Pref. 3-X-2011, collector unknown (NSMT-Ar 12559).

Other specimens examined. 27♀13♂ from Aomori, Iwate, Miyagi, Akita, Niigata, Ibaraki, Tochigi, Saitama, Tokyo, Kanagawa, Yamanashi, Shizuoka, Mie, Tottori, Shimane, Hiroshima, Kagawa, Ehime and Fukuoka Prefectures.

Specimens examined for comparison. Syntypes of *Cyrtarachne inaequalis*, 1♀, Jonghoo, 1juv., Tharawaddy, Burma, Oates leg. (NHM 1895.9.21.654-655).

Etymology. The specific name is dedicated to Mr. Akira Shinkai, Tokyo, who is studying about web building behavior and web structure of this genus.

Diagnosis. Female of this species resembles *Cyrtarachne inaequalis* thorell 1895 in general appearance, but can be separated by the much longer scape of epigyne (Figs. 9–10) than the latter species (Figs. 7–8). This species also resembles *C. jucunda* n. sp., but can be distinguished from the latter by the following points: (1) scape of epigyne is much wider (Figs. 9–10) than the latter (Figs. 11–12), (2) median apophysis of male palp much larger (Fig. 13) than the latter (Fig. 14).

Description. Based on holotype ♀ and paratype 1♂ (NSMT-Ar 12557, 12562). Coloration and markings. Female (Fig. 3): carapace yellowish brown, dorsum of abdomen whitish yellow, with a pair of dark color markings on shoulder. Male (Fig. 4): carapace reddish brown, dorsum of abdomen whitish yellow, anteriorly darker.

Measurements. ♀/♂, measurements in parentheses indicate the range among type series. Body 10.92 (6.60–11.69)/2.64 (2.25–3.08) long. Carapace 3.56 (2.64–3.84)/1.30 (1.20–1.38) long; 3.48 (2.64–3.80)/1.15 (1.10–1.25) wide. Length of legs [tarsus＋metatarsus＋tibia＋patella＋femur＝total]: I, 0.68＋1.84＋2.28＋1.56＋3.16＝9.52/0.39＋0.84＋0.98＋0.55＋1.44＝4.20; II, 0.64＋1.74＋2.16＋1.50＋3.00＝9.04/0.38＋0.78＋0.90＋0.55＋1.30＝3.91; III, 0.60＋1.08＋1.22＋0.96＋1.96＝5.82/0.33＋0.49＋0.50＋0.36＋0.88＝2.56; IV, 0.56＋1.48＋1.88＋1.20＋2.80＝7.92/0.30＋0.58＋0.68＋0.44＋1.06＝3.06. Abdomen 8.00 (5.30–9.54)/1.53 (1.33–1.73) long; 9.30 (6.40–11.85)/1.88 (1.65–2.08) wide.

Female (Fig. 3). Carapace almost as long as wide [length divided by width 1.02 (0.99–1.04)]. Median ocular area wider than long [length divided by width 0.81 (0.75–0.82)]; almost as wide in front as behind [anterior width divided by posterior width 1.00 (0.95–1.05)]. Labium wider than long [length divided by width 0.67 (0.60–0.67)]. Sternum almost as wide as long [length divided by width 0.96 (0.96–1.02)]. Length of leg I divided by length of carapace 2.67 (2.64–2.96). Metatarsus and tibia of 1st and 2nd legs prolaterally with a row of weak spines. Abdomen wider than long [length divided by width 0.86 (0.81–0.88), scape of epigyne long inverted triangle in ventral view (Fig. 9).

Male (Fig. 4). Carapace longer than wide [length divided by width 1.13 (1.09–1.13)]. Median ocular area wider than long [length divided by width 0.79 (0.77–0.88)]; slightly wider in front than behind [anterior width divided by posterior width 1.12 (1.08–1.13)]. Labium wider than long [length divided by width 0.64 (0.60–0.67)]. Sternum almost as wide as long [length divided by width 1.00 (0.94–1.00)]. Length of leg I divided by length of carapace 3.23 (3.06–3.23). Metatarsus and tibia of 1st and 2nd legs prolaterally with a row of spines. Palp (Fig. 13): embolus rostriform, median apophysis wide, cymbium posteriorly with distinct depression. Abdomen wider than long [length divided by width 0.81 (0.79–0.83).

Distribution. Japan (Honshu, Shikoku, Kyushu, and Nansei Isls.), Taiwan, China.

Cyrtarachne jucunda n. sp.
[Japanese name: Magii-torinofun-damashi]
(Figs. 5–6, 11–12, 14)

Type series. Holotype: ♀, Haneji, Nago-shi, Okinawa-jima Is., Okinawa Pref., Japan, 19-XI-2011, N. Ooseko leg. (NSMT-Ar 12567). Paratypes: 1♂, 15-VII-2007 (NSMT-Ar 12571), 2♂, 12-IX-2010 (NSMT-Ar 12572, 12573), 1♀, 19-IX-2010 (NSMT-Ar 12568), 1♂, 4-IX-2011 (NSMT-Ar 12569), 1♂, 10-IX-2011 (NSMT-Ar 12570), the same locality as the holotype, M. Sugimoto leg.

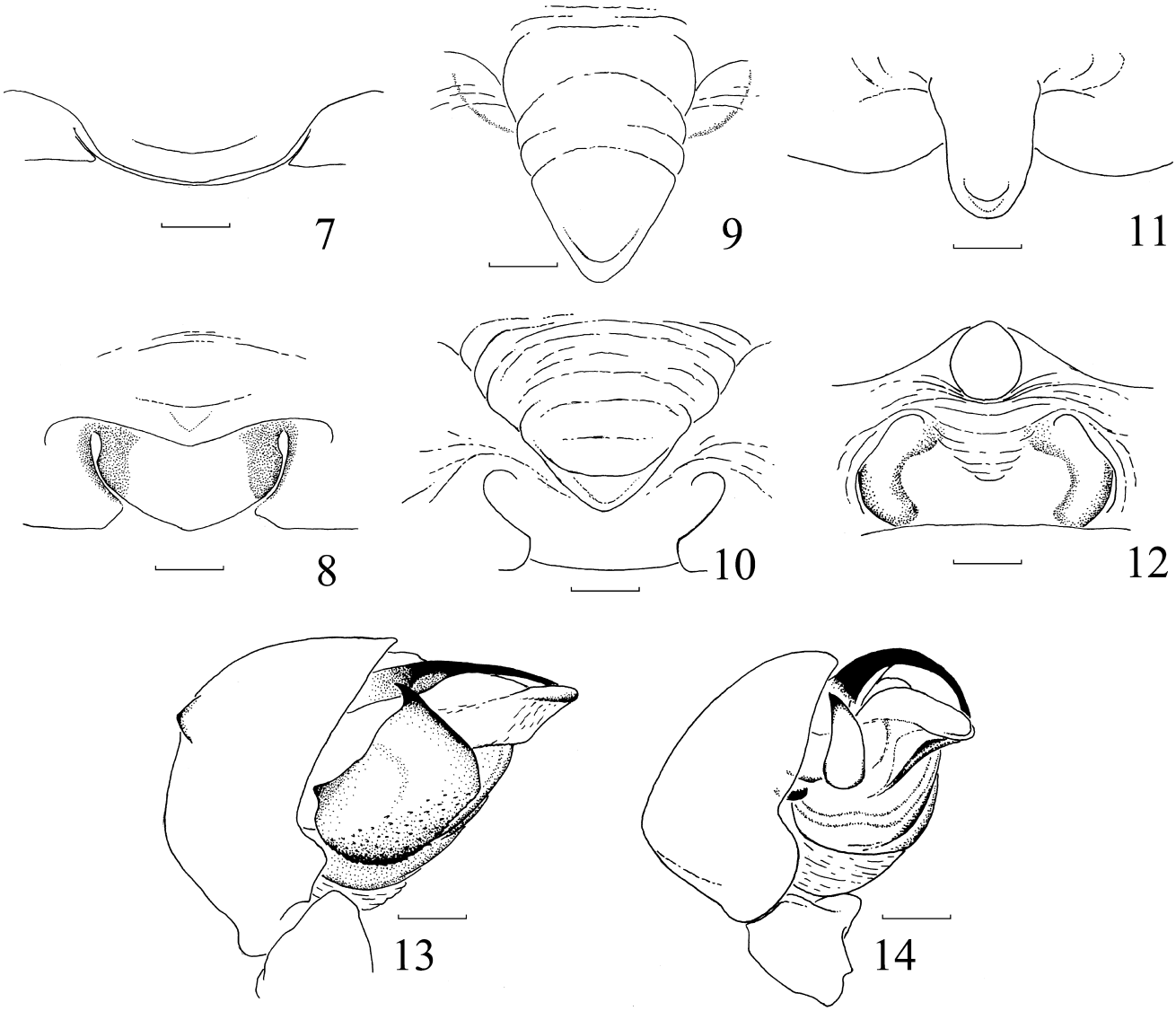
Specimens examined for comparison. Syntypes of *Cyrtarachne inaequalis*, 1♀, Jonghoo, 1juv., Tharawaddy, Burma, Oates leg. (NHM 1895.9.21.654–655).

Etymology. The specific name is derived from its lovely general appearance.

Diagnosis. Female of this species closely resembles those of *Cyrtarachne inaequalis* thorell 1895 and *C. akirai* n. sp. in general appearance, but can be separated from them by the tongue shaped scape of epigyne (Figs. 7–12). The male of the present new species can be separated from that of *C. akirai* n. sp. by the curved embolus and small median apophysis of male palp (Figs. 13–14).

Description. Based on holotype ♀ and paratype 1♂ (NSMT-Ar 12567, 12570). Coloration and markings. Female (Fig. 5): carapace pale brown, dorsum of abdomen yellowish brown, with a pair of dark color markings on shoulder. Male (Fig. 6): carapace reddish brown, dorsum of abdomen reddish brown, posteriorly yellow.

Measurements. ♀/♂, measurements in parentheses indicate the female paratype or range among male paratypes. Body 7.70 (8.90)/2.64 (2.30–3.08) long. Carapace 3.40 (3.48)/1.28 (1.13–1.40) long; 3.28 (3.40)/1.13 (1.01–1.23)



Figs. 7–14. Genital organ. 7–8, *Cyrtarachne inaequalis* (syntype: NHM 1895.9.21.654–655); 9–10, 13, *C. akirai* n. sp. (9–10, holotype, NSMT-Ar 12557; 13, paratype, NSMT-Ar 12563); 11–12, 14, *C. jucunda* n. sp. (11–12, holotype, NSMT-Ar 12567; 14, paratype, NSMT-Ar 12570); 7, 9, 11, epigyne, ventral view; 8, 10, 12, same, posterior view; 13–14, male palp, prolateral view. Scales＝0.1 mm.

wide. Length of legs [tarsus＋metatarsus＋tibia＋patella＋femur＝total]: I, 0.60＋1.76＋2.10＋1.44＋2.94＝8.84/0.35＋0.70＋0.83＋0.53＋1.28＝3.69; II, 0.60＋1.60＋1.92＋1.44＋2.74＝8.30/0.35＋0.68＋0.78＋0.50＋1.15＝3.46; III, 0.48＋0.96＋1.04＋0.92＋1.76＝5.16/0.33＋0.43＋0.48＋0.38＋0.74＝2.36; IV, 0.52＋1.36＋1.70＋1.14＋2.54＝7.26/0.30＋0.50＋0.63＋0.41＋0.95＝2.79. Abdomen 6.25 (7.35)/1.58 (1.43–1.88) long; 8.05 (8.90)/2.00 (1.75–2.25) wide.

Female (Fig. 5). Carapace almost as long as wide [length divided by width 1.04 (1.02)]. Median ocular area wider than long [length divided by width 0.83 (0.82)]; almost as wide in front as behind [anterior width divided by posterior width 1.02 (1.05)]. Labium wider than long [length divided by width 0.71 (0.57)]. Sternum slightly wider than long [length divided by width 0.94 (0.96)]. Length of leg I divided by length of carapace 2.60 (2.59). Metatarsus and tibia of 1st and 2nd legs prolaterally with a row of weak spines. Abdomen wider than long [length divided by width 0.78 (0.83), scape of epigyne narrow tongue shaped (Figs. 11–12).

Male (Fig. 6). Carapace longer than wide [length divided by width 1.13 (1.11–1.14)]. Median ocular area wider than long [length divided by width 0.71 (0.71–0.85)]; wider in front than behind [anterior width divided by posterior width 1.31 (1.18–1.31)]. Labium wider than long [length divided by width 0.55 (0.50–0.59)]. Sternum almost as wide as long [length divided by width 0.96 (0.96–1.12)]. Length of leg I divided by length of carapace 2.88 (2.82–3.00). Metatarsus and tibia of 1st and 2nd legs prolaterally with a row of spines. Palp (Fig. 14): embolus curved, median apophysis small. Abdomen wider than long [length divided

by width 0.79 (0.79−0.83).

Distribution. Japan (Okinawajima Is.).

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Appendix. DDBJ/EMBL/GenBank accession numbers of sequence data analyzed in this study. Male in question and female from Okinawajima Is. were described as *Cyrtarachne jucunda* n. sp. and females from the Mainland were described as *C. akirai* n. sp. in this paper.

Species	Locality	Accession No.
Male in question 1	Haneji, Okinawajima Is.	AB820893
Male in question 2	Haneji, Okinawajima Is.	AB820894
Female from Okinawajima	Haneji, Okinawajima Is.	AB820895
Female from Mainland 1	Jinseki-cho, Hiroshima Pref.	AB820888
Female from Mainland 2	Jinseki-cho, Hiroshima Pref.	AB820889
Female from Mainland 3	Yokosawa-iri, Tokyo Pref.	AB820890
Female from Mainland 4	Oda-shi, Shimane Pref.	AB820891
Female from Mainland 5	Masuho-cho, Yamanashi Pref.	AB820892
Cyrtoarachne bufo	Masuho-cho, Yamanashi Pref.	DQ518421
Cyrtoarachne bufo	Saga-shi, Saga Pref.	AB820871
Cyrtoarachne bufo	Jinseki-cho, Hiroshima Pref.	AB820873
Cyrtoarachne bufo	Jinseki-cho, Hiroshima Pref.	AB820872
Cyrtoarachne bufo	Orikisawa, Chiba Pref.	AB820874
Cyrtarachne yunoharuensis	Ome-shi, Tokyo Pref.	AB46975
Cyrtarachne yunoharuensis	Komaki-shi, Aichi Pref.	AB820879
Cyrtarachne yunoharuensis	Oda-shi, Shimane Pref.	AB820880
Cyrtarachne nagasakiensis	Iriomotejima Is., Okinawa Pref.	AB820875
Cyrtarachne nagasakiensis	Tokunoshima Is., Kagoshima Pref.	AB820878
Cyrtarachne nagasakiensis	Takarajima Is., Kagoshima Pref.	AB820876
Cyrtarachne nagasakiensis	Takarajima Is., Kagoshima Pref.	AB820877
Paraplectana tsushimensis	Okinawajima Is., Okinawa Pref.	AB627006
Paraplectana tsushimensis	Okinawajima Is., Okinawa Pref.	AB627003
Paraplectana tsushimensis	Tsushima Is., Nagasaki Pref.	AB627004
Paraplectana tsushimensis	Takarajima Is., Kagoshima Pref.	AB627005
Paraplectana tsushimensis	Okinawajima Is., Okinawa Pref.	DQ518422
Paraplectana tsushimensis	Amami-oshima Is., Kagoshima Pref.	DQ518423
Paraplectana sakaguchii	Hachinohe-shi, Aomori Pref.	AB627002
Paraplectana sakaguchii	Hinode-machi, Tokyo Pref.	AB46976
Paraplectana sakaguchii	Goto-shi, Nagasaki Pref.	DQ518420
Ordgarius hobsoni	Uchiko-cho, Ehime pref.	DQ518417
Ordgarius hobsoni	Okinawajima Is., Okinawa Pref.	AB820881
Ordgarius hobsoni	Higashi-matsuyama-shi, Saitama Pref.	AB820882
Ordgarius sexspinosus	Masuho-cho, Yamanashi Pref.	DQ518418
Ordgarius sexspinosus	Masuho-cho, Yamanashi Pref.	AB820884
Ordgarius sexspinosus	Masuho-cho, Yamanashi Pref.	AB820885
Ordgarius sexspinosus	Misugi-mura, Mie Pref.	AB820883
Pasilobus hupingensis	Okinawajima Is., Okinawa Pref.	DQ518424
Pasilobus hupingensis	Ureshino-inoue-cho, Mie Pref.	DQ518425
Pasilobus hupingensis	Okinawajima Is., Okinawa Pref.	AB820887
Pasilobus hupingensis	Osaka Pref.	AB820886